Applicant: Boyle, et al. U.S. Application No. 09/676,135

Appendix A: marked up version showing the changes made in the specification

In the Specification:

A polypeptide was predicted to be encoded by SEQ ID NO: 2 as set forth below. The polypeptide was predicted using a software program called FASTY (available from the Internet website at: fasta.bioch.virginia.edu [http://fasta.bioch.virginia.edu]) which selects a polypeptide based on a comparison of translated novel polynucleotide to known polypeptides (W.R. Pearson, Methods in Enzymology, 183: 63-98 (1990), herein incorporated by reference).

Applicant: Boyle, et al. U.S. Application No. 09/676,135

Appendix C - Clustal W Analysis

- 12 - HYS-28

Alignment Report of Align_Carboxypeptidase.meg ClustalW (Slow/Accurate, Gonnet) Sunday, July 28, 2002 1:04 PM

Page 1

```
----- 21489916 CPO
1
   -- MLAFLILVTVTLASAHHS CAB46991
1
  M K L H G L G I L V A I I L Y E Q H - - gi7416967 Mouse TAFI
1
  MKLHGLGILVAIILYEQH - - BAB03402 Mouse
  GMLVPGG - - - - - - - - LD 00002227Fa161 aa1
12
1
   GEHFEGEKVFRVNVEDENDI CAB46991
19
  GFAFQSGQVLSALPRTSRQV gi7416967 Mouse TAFI
19
  GFAFQSGQVLSALPRTSRQV BAB03402 Mouse
19
  GYDRSLAQHRQEIVDKSVSP 00002227Fa161 aa1
   ---<u>S</u>LA<u>QH</u>RQ<u>EIVD</u>K<u>SV</u>S<u>P</u> 21489916_CPO
  SLLHELASTRQIDFWKPDSV CAB46991
   QLLQN|L|TTTYEVVLWQP|V|TA gi7416967_Mouse_TAFI
39
  QLLQNLTTTYEVVLWQPVTA BAB03402 Mouse
  WSLETYS - - - - - - - - - 00002227Fa161_aa1
40
  WSLETYS - - - - - - - 21489916 CPO
17
  TQIKPHSTVDFRVKAEDILA CAB46991
59
  EFIEKKKEVHFFVNASDVDS gi7416967_Mouse_TAFI
59
  EFIEKKKEVHFFVNASDVDS BAB03402_Mouse
59
47
   -----00002227Fa161 aa1
24
   ----- 21489916 CPO
79 VEDFLEQNELQYEVLINNLR CAB46991
79
  VKAHLNVSRIPFNVLMNNVE gi7416967_Mouse_TAFI
79
  V K A H L N V S R I P F N V L M N N V E BAB03402 Mouse
   ---- 00002227Fa161 aa1
47
24
   99
  SVLEAQ-FDSRV--RTTGHS CAB46991
99
  DLIEQQTFNDTVSPRASASY gi7416967_Mouse_TAFI
  DLIEQQTFNDTVSPRASASY BAB03402 Mouse
   - - | I Y H P M G E I Y E W M R E I S E K | 00002227Fa161_aa1
49
   - - <u>I Y H P M G E I Y E W M R E I S E K</u> 21489916 CPO
116 Y E K Y N N W E T I E A W T K Q V T S E CAB46991
119 Y E Q Y H S L N E I Y S W I E V I T E Q gi7416967 Mouse TAFI
119 Y E Q Y H S L N E I Y S W I E V I T E Q BAB03402 Mouse
```

Sunday, July 28, 2002 1:04 PM Y K E V V T Q H F L G V T Y E T H P I Y 00002227Fa161 aa1 YKEVVTQHFLGVTYETHPMY 21489916_CPO 136 NPDLISRTAIGTFLGNNIY CAB46991 139 H P D M L Q K I Y I G S S F E K Y P L Y gi7416967 Mouse TAFI 139 H P D M L Q K I Y I G S S F E K Y P L Y BAB03402 Mouse Y L K I S Q P S G N P K K I I W M D C G 00002227Fa161 aa1 87 Y L K I S Q P S G N P K K I I W M D C G 21489916 CPO 156 L|L K|V - G K P|G|P N|K|P A|I|F|M D C G| CAB46991 159 V L K V S G K E Q R I K N A I W I D C G gi7416967_Mouse_TAFI 159 VLKVSGKEQRIKNAIWIDCG BAB03402 Mouse 107 | I H A R E W I A P A F C Q W F V K E I L | 00002227Fa161 aa1 84 I HAREWI<u>AP</u>AFCQWFV<u>K</u>E<u>IL</u> 21489916 CPO 175 F H A R E W I S H A F C Q W F V R E A V CAB46991 179 I H A R E W I S P A F C L W F I G Y V T gi7416967 Mouse TAFI 179 I H A R E W I S P A F C L W F I G Y V T BAB03402 Mouse 127 | Q N H K D N S R I R K L L R N L D F Y V | 00002227Fa161_aa1 104 Q N H K D N S S I R K L L R N L D F Y V 21489916 CPO 195 LTYGYESHMTEFLNKLDFYV CAB46991 199 QFHGKENLYTRLLRHVDFYI gi7416967 Mouse TAFI 199 QFHGKENLYTRLLRHVDFYI BAB03402 Mouse 147 LPVLNIDGYIYTWTTDRLWR 00002227Fa161 aa1 124 LPVLNIDGYIYTWTTDRLWR 21489916 CPO 215 <u>L P V L N I D G Y I Y T W T K N R M W R</u> CAB46991 219 M P V M N V D G Y D Y T W K K N R M W R gi7416967_Mouse_TAFI 219 MPVMNVDGYDYTWKKNRMWR BAB03402 Mouse 167 KSRSPHNNGTCFGTDLNRNF 00002227Fa161 aa1 144 | K S R S P H N N G T C F G T D L N R N F | 21489916 CPO 235 KTRSTNAGTTCIGTDPNRNF CAB46991 239 KNR SAHKNNRCVGTDLNRNF gi7416967 Mouse TAFI 239 KNRSAHKNNRCVGTDLNRNF BAB03402 Mouse 187 N A S - W C S I G A S R N C Q D Q T F C 00002227Fa161_aa1 164 NAS - WCSIGASRNCQDQTFC 21489916 CPO 255 DAG - W CTTGASTDPCDETYCCAB46991

259 A S K H W C E K G A S S S S S S E T Y C gi7416967_Mouse_TAFI

259 A S K H W C E K G A S S S S C S E T Y C BAB03402 Mouse

Sunday, July 28, 2002 1:04 PM

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206 | G T G P V S E P E T K A V A S F I E S K | 00002227Fa161_aa1
183 G T G P V S E P E T K A V A S F I E S K 21489916 CPO
274 GSAAESEKETKALADFIRNN CAB46991
279 |G|L Y|P|E|S E P E|V|K A V A|D|F|L R R N gi7416967_Mouse_TAFI
279 GLYPESEPEVKAVADFLRRN BAB03402_Mouse
226 | K D D I L C F L T M H S Y G Q L I L T P | 00002227Fa161_aa1
203 KDDILCFLTMHSYGQLILTP 21489916_CPO
294 L S S |I|KAYLTI|HSY|S|Q|M|IL|Y|P|CAB46991
299 I|D|H|I|KAYIS|MHSY|S|Q|Q|IL|F|P|gi7416967_Mouse_TAFI
299 IDHIKAYISMHSYSQQILLFP BAB03402_Mouse
246 | Y G Y T K N K S S N H P E M I Q V G Q K | 00002227Fa161_aa1
223 Y G Y T K N K S S N H P E M I Q V G Q K 21489916_CPO
314 | Y | S | Y | D Y K <u>L P</u> E | N N A | E | L N N L A K A CAB46991
319 |Y|S|Y|NRS|KS|KD|H|E|E|LSL|V|ASE gi7416967_Mouse_TAFI
319 YSYNRSKSKDHEELSLVASE BAB03402_Mouse
266 | A A N A L K A K Y G | - | T N Y R V G S S A | 00002227Fa161_aa1
243 A A N A L K A K Y G - T N Y R V G S S A 21489916_CPO
334 |A|V K E|L|A T L|Y G|-|T|K|Y|T Y|G|P G|A| CAB46991
339 |A|VR|A|IESINKN|T|R|Y|TH|GS|GS gi7416967_Mouse_TAFI
339 AVRAIESINKNTRYTHGSGS BAB03402_Mouse
285 | D I L Y A S S G S S R D W A R D I G I P | 00002227Fa161_aa1
262 DILYASSGSSRDWARDIGIP 21489916_CPO
353 TT I Y P A A G G S D D W A Y D Q G I K CAB46991
359 ES|LY|LAP|G|G|S|D|DW|IY|D|L|GI|K gi7416967_Mouse_TAFI
359 ESLYLA PGGSDDWIYDLGIK BAB03402_Mouse
305 | F S Y T F E L R D S G T Y G F V L P E A | 00002227Fa161_aa1
282 F S Y T F E L R D S G T Y G F V L P E A 21489916_CPO
373 Y|S|F|TFELRD|K|G|R|YGF|I|LPE|S CAB46991
379 Y|S|F|T|I|E|L|R|D|T|G|R|Y|G|F|L|L|P|E|R|gi7416967_Mouse_TAFI
379 YSFTIELRDTGRYGFLLPER BAB03402_Mouse
325 | Q I Q P T C E E T M E A V L S V L D D V | 00002227Fa161_aa1
302 Q I Q P T C E E T M E A V L S V L D D V 21489916_CPO
393 QIQATCEETMLAIKYVTNYV CAB46991
399 Y \mid I \mid K \mid P \mid T \mid C \mid A \mid E \mid A \mid L \mid A \mid A \mid I \mid S \mid K \mid V \mid W \mid H \mid V \mid gi7416967 Mouse_TAFI
399 Y I K P T C A E A L A A I S K I V W H V BAB03402_Mouse
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Sunday, July 28, 2002 1:04 PM

| 345 | Y | Α | K | Н | W | Η | S | D | S | Α | G | R | V | T | S | Α | Т | M | L | L | 00002227Fa161_aa1 |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------------------|
| 322 | Y | Α | K | Н | W | Н | S | D | S | A | G | R | V | Т | S | Α | Т | M | L | L | 21489916_CPO |
| 413 | L | G | Н | L | | | | | | | | | | | | | | | | | CAB46991 |
| 419 | I | R | N | Т | | | | | | | | | | | | | | | | | gi7416967_Mouse_TAFI |
| 419 | I | R | N | Т | | | | | | | | | | | | | | | | | BAB03402_Mouse |
| | | | | | | | | | | | | | | | | | | | | | |
| 365 | G | L | L | V | S | С | M | S | L | L | | | | | | | | | | | 00002227Fa161_aa1 |
| 342 | G | L | L | V | S | С | M | S | L | L | | | | | | | | | | | 21489916_CPO |
| 416 | | | | | | | | | | | | | | | | | | | | | CAB46991 |
| 422 | | | | | | | | | | | | | | | | | | | | | gi7416967_Mouse_TAFI |
| 422 | | | | | | | | | | | | | | | | | | | | | BAB03402_Mouse |

Decoration 'Decoration #1': Box residues that match 00002227Fa161_aa1 exactly.

KEY

For convenience, all numbering refers to the numbering of 00002227Fa161_aa1.

Bolded Residues (Y50 through E332) = Zinc carboxypeptidase Pfam signature (PF00246).

Barred Residues (K99 through F121) = Residues below the bar comprise a PROSITE Carboxypept_Zn_1 signature (PS00132)
PS00132 signature = [PK]-x-[LIVMFY]-x-[LIVMFY]-x-(4)-H-[STAG]-x-E-x-[LIVM]-[STAG]-x(6)- [LIVMFYTA]
(http://www.expasy.ch/prosite).

Asteriks (*) = Residues indicated by an asterisk are those involved in the coordination of the active site zinc atom and are highly conserved amongst members of the CPA/B carboxypeptidase subfamily.

(JBC 2002; 277 pp. 14954-64)

Dots (.) = Residues indicated by a dot are those that are important for substrate binding and catalysis and are highly conserved amongst members of the CPA/B carboxypeptidase subfamily.

(JBC 2002; 277 pp. 14954-64)